THE PLASMA LEVEL OF PROPROTEIN CONVERTASE FURIN IN PATIENTS WITH SUSPECTED INFECTION IN THE EMERGENCY ROOM; A PROSPECTIVE COHORT STUDY

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Infektiotaudit ovat tavallinen ongelma päivystysklinikalla. Erityisesti sepsiksen diagnosointi on hankalaa, sillä taudille ei tällä hetkellä ole olemassa sopivaa biomarkkeria. Sepsis on melko yleinen, nopeasti etenevä systeeminen infektio, johon liittyy merkittävän suuri kuolleisuus. Jo parissa päivässä oireiden alkamisesta kehittyvään vakavaan sepsikseen menehtyy noin 70 % potilaista. Tämän vuoksi varhaisen vaiheen diagnostiikka ja nopea, tehokkaan mikrobilääkehoidon aloitus ovat elintärkeitä sepsispotilaille. Biomarkkerin puuttumisen vuoksi hoidon aloitus viivästyy ja potilaan ennuste heikkenee.

Aiemmissa tutkimuksissa on havaittu elimistössä laaja-alaisesti esiintyvän proproteiini konvertaasi furiinin yhteys monenlaisiin tulehdustiloihin. Furiini on proteaasientsyymi, joka säätelee muun muassa T auttaja 1 -solujen aktiivisuutta sekä TLR7 reseptorien määrää. Lisäksi lipopolysakkaridi (LPS) lisää sen ilmentymistä makrofageissa.

Tutkimuksen tavoitteena on selvittää voitaisiinko päivystyksessä potilaiden plasmanäytteistä mitattuja furiinipitoisuuksia käyttää hyväksi infektion varhaisen vaiheen diagnostiikassa, vakavuuden arvioinnissa, kuolleisuuden ennustamisessa ja/tai oikeanlaisen antibioottihoidon valitsemisessa.

Potilasaineisto kerättiin Satakunnan keskussairaalasta vuosina 2004 ja 2005. Se koostuu täysi-ikäisistä, päivystyspoliklinikalle saapuneista infektioepäilypotilaista. Verinäytteet kerättiin päivystyksessä ja muu data haastattelusta ja sairaskertomuksista. Plasman furiinipitoisuus määritettiin kaupallisella ELISA-menetelmällä (Human Furin Enzyme Linked Immunosorbent Assay, Sigma-Aldrich®) ja tulokset jaettiin korkean ja matalan pitoisuuden ryhmiin. Myös potilaat jaoteltiin infektiotyypin perusteella viiteen diagnostiseen ryhmään. Tilastolliset analyysit suoritettiin SPSS-ohjelmalla (IBM, versio 22).

Tilastollisesti merkitsevää yhteyttä ei havaittu verrattaessa sepsiksen yleisyyttä ($P = 0,421$), diagnostista ryhmää ($P = 0,737$) sekä veriviljelyn tulosta ($P = 0,351$) potilaan furiinitasoon. Furiinipitoisuus ja kuolleisuus eivät myöskään liittyneet toisiinsa ($P = 0,898$). Merkitsevät korrelaatiot löydettiin korkean furiinitason ja tupakoimattomuuden ($P = 0,034$) sekä reumasairauksien ($P < 0,001$) väliltä.

Furiini ei sovellu infektiomarkkeriksi päivystyspoliklinikalla eikä sen plasmapitoisuudesta voida tehdä johtopäätöksiä liittyen taudin vakavuuteen tai potilaan ennusteeseen. Pitoisuuksien määrittämisestä ei myöskään ole apua oikean mikrobilääkkeen valinnassa. Toisaalta, furiinitasojen jakautumista olisi jatkossa mielenkiintoista tutkia erityisesti reumapotilaista koostuvassa aineistossa.

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1. INTRODUCTION

Sepsis is a major cause of mortality. In severe sepsis, mortality rises up to 60-70% [1, 2]. Potentially fast disease progression is the main reason why early diagnosis and quick adequate treatment are extremely important in this condition. Even an hour-long delay in adequate antimicrobial treatment may have an adverse impact on prognosis [3, 4].

Currently used biomarkers for the diagnosis of sepsis are C-reactive protein (CRP), blood leukocytes, and procalcitonine (PCT) [5, 6]. None of these biomarkers are suitable for the early stages of sepsis; they can be used for confirmation, but not for exclusion of the diagnosis. For example, CRP levels begin to rise only after 12 hours of the early signs of sepsis and it is not a specific marker of bacterial infection [7, 8].

The proprotein convertase subtilisin/kexin enzymes (PCSKs) belong to the subtilisin superfamily of serine endoproteases, which convert immature precursor proteins into the biologically functional units by catalyzing post-translational site-specific hydrolytic cleavage [9, 10]. FURIN is the first found and therefore the most studied mammalian proprotein convertase. This ubiquitously expressed enzyme is located mainly in the trans-Golgi network (TGN) of the secretory pathway. In addition, FURIN cycles between TGN and the cell surface via the endosomal system, and a proportion of it is secreted to the extracellular space [11, 12]. Because FURIN is widely expressed, its activity regulates the maturation of numerous precursor proteins. Its substrates include various growth factors and their receptors, enzymes, hormones, cytokines, serum proteins, as well as extracellular-matrix proteins [10, 11]. In addition to FURIN´s crucial function in the maintenance of cellular homeostasis, it is also involved in the pathogenesis of several diseases. For example, increased FURIN levels are associated with aggressive cancers and metastatic activity [11], and it is highly expressed in chronically inflamed tissues in rheumatoid arthritis (RA) and atherosclerosis [13, 19]. FURIN has also a significant role in the activation of viruses and bacterial pro-toxins. These pathogens include bird flu (AH5N1), HIV and Ebola viruses as well as the toxins of *Bacillus anthracis* and *Clostridium botulinum* [12].

Because FURIN is essential for the embryogenesis, our understanding on its cell-type specific *in vivo* function is incomplete [13]. However, we have previously shown that FURIN is profoundly upregulated in T helper type 1 (Th1) cells via the IL-12/Stat4 pathway and by T cell receptor mediated signaling [14]. T-cell-expressed FURIN regulates the Th cell polarization and the peripheral immune tolerance primarily by controlling the functional maturation of anti-inflammatory cytokine transforming growth factor beta (TGFβ-1) [14, 15, 16]. In macrophages the expression and secretion of

FURIN is induced by lipopolysaccharide (LPS) [17, 18], and a FURIN-like enzyme controls the quantity and activation of the human Toll-like receptor 7 (TLR7) [20]. In contrast, relatively little is known about the FURIN levels in human infections, but an elevated serum concentration has been reported in chronically *Salmonella typhi* infected patients [17].

The fact that FURIN is upregulated upon immune cell activation and its key regulatory role in host defence prompted us to evaluate the usability of plasma FURIN as an early stage infection biomarker. Specifically, we analyzed whether the proprotein convertase FURIN levels in plasma could be useful in predicting the severity and case fatality of infection in a large, randomly selected cohort of patients that were admitted the emergency room with suspected infection.

2. METHODS

2.1. Study population

The study population was recruited in the Satakunta Central Hospital, Finland within a 14-month study period in 2004 to 2005 [6, 21, 22, 23]. Satakunta Central Hospital is a secondary care hospital with 350-beds serving a population of 240 000 inhabitants. There are no other hospitals with an emergency room and an intensive care unit (ICU) in the Satakunta area. All of the involved patients were adults, who had been admitted to the emergency room with a suspected infection status and been drawn a blood culture sample. The study was approved by the ethical review board of the Satakunta Hospital District. Written consent was obtained from patients or close relatives. To ensure written informed consent and interview within 24-48 hours, only patients admitted between Sunday 7 a.m. and Wednesday 3 p.m. were enrolled [23].

The plasma samples were taken simultaneously with the blood culture samples and collected in two 10-ml EDTA tubes. The EDTA tubes were kept on ice before the centrifugation in 2500 G-force for 10-15 minutes. The samples were then transported in 1 to 2 ml aliquots to CryoPure® (Sartsedt, Germany) tubes and further stored at -70 °C [23].

The information for the statistical analysis was obtained from an interview held by a researcher or a research nurse within 24-48 hours after the admission. Highest body temperature, lowest blood pressure, highest pulse and the respiratory rate were measured daily during the first week of hospitalization. In addition, symptoms and clinical signs, Glasgow coma scale, risk factors for sepsis, underlying disease, diagnosis at admission, prospective organ failure (cardiovascular, hematological, hepatic, renal, respiratory or central nervous system), universal case fatality and the case fatality caused by sepsis were documented in ICU and in the ward. Other information such as the potential reason and the cause of the infection and trauma together with the final diagnose were checked from

patient´s medical records. Follow-up surveys were conducted by phone 3 months and one year after patient´s admission. Before initiating the study, a pre-evaluation of the target population was conducted to ensure the representativeness of the cohort. This assessment covered 1551 consecutive patients from whom blood had been collected in the emergency department for blood culture. The rate of positive blood culture in the pre-evaluation was 8.3% and case fatality by day 28 after admission was 6.7%. No significant differences in age, gender, rate of positive blood culture or mortality were noted between patients admitted on study days and those admitted on other days, or between the study and the pre-evaluation populations. [23]

In total, blood samples were taken from 609 patients. 55 samples were removed because of the patient´s reluctance to participate in the research, and 17 samples were left out from other reasons: 1 missing, 11 unclear results, 3 with SIRS and multi-organ failure but no bacterial infection and 2 samples ran out. As a result, the final cohort comprised of 537 patients [23].

2.2. Measurement of plasma FURIN levels

The concentration of proprotein convertase FURIN was determined from EDTA plasma samples using a commercial Human FURIN Enzyme-Linked Immunosorbent Assay (ELISA) Kit (Sigma-Aldrich®, USA) according to manufacturer´s instructions. The reliable detection limit of the assay was determined to be 370 pg/ml, and the concentrations below that were considered as low/absent. In general, all samples were analyzed once, but analysis was replicated with a higher dilution for the samples showing exceptionally high FURIN levels. The patients were stratified into low and high plasma FURIN groups with a cut-off at 370 pg/ml.

2.3. Statistical analysis

The detected FURIN concentrations were transferred to the SPSS Statistics for Windows software (IBM, version 22) for statistical analyses. The study population was divided into five diagnosis groups based on The American Collage of Chest Physicians/Society of Critical Care Medicine Consensus Conference definitions of the severity of the infection stage. [24].

Nonparametric independent-samples t-test for Independent Samples or Analysis of Variance were used with normally distributed variables, and Mann-Whitney U-Test or Kruskal-Wallis test for the rest. Two binomial variables were analyzed with Chi-Square or Fishers exact test. Linear and Logistic Regression were used to examine the effect of potential confounders. The Kaplan-Meier and the Log-rank test were used to investigate the connection between the FURIN levels and the case fatality.

The common significance levels were used to determine the importance of the outcomes: $P < 0.05$ illustrated a statistically significant result, and $P < 0.001$ was considered as a statistically highly significant discovery.

3. RESULTS

The demographic data of the study population (n = 537), underlying diseases as well as the infection-associated clinical parameters during the first 28 days are presented in Table 1. The plasma FURIN levels were measured from all samples as described in methods. The assay standard curve was prepared using serial dilutions of recombinant FURIN protein, and a reliable linearity was detected only with FURIN levels above 370 pg/ml, which was thus determined as cut-off for high FURIN levels in plasma. The cohort was stratified into five study groups on the basis of ACCP/SCCM Consensus Conference definitions (Table 2) [24], and further divided into low and high FURIN samples. In the five different groups high FURIN levels were detected in 14.9% - 22.0% of samples, with lowest percentage of high FURIN samples in Bacterial infection, no SIRS group (Group 2), and highest in no SIRS, no bacterial infection group (Group 1). However, differences in the prevalence of high FURIN levels between different groups were not significant ($P = 0.737$).

Table 1 *Demographic and clinical characteristics of the study population (N = 537).*

	N	Percentage
Characteristic		
Age, median (range)	64.2 (18 - 100)	
Gender (male)	310	(57.7%)
Obesity (BMI[a] ≥ 30 kg/m^2), median (range)	27.0 (14.7 - 67.6)	
Alcohol abuse[b]	25	(4.7%)
Smoking (current smoker)	126	(23.5%)
Diabetes (types 1 and 2)	81	(15.1%)
Solid cancer	78	(14.5%)
Malignancy (solid or haematological)	95	(17.7%)
Rheumatic disease	50	(9.3%)
Chronic renal insufficiency[c]	18	(3.4%)
Cardiovascular disease[d]	289	(53.8%)
Continuous cortisone treatment[e]	59	(11.0%)
Clinical parameters (d 0-28)		
Case fatality (d 28)	33	(6.1%)
Case fatality (d 90)	58	(10.8%)
Case fatality (1 year)	112	(20.9%)
ICU[f] stay needed	42	(7.8%)
Hypotension[g]	28	(5.2%)
DIC[h]	8	(1.5%)
Decreased GCS[i]	60	(11.2%)
Mechanical ventilation	14	(2.6%)
C-PAP/bi-PAP[j]	22	(4.1%)
Sepsis + organ dysfunction	49	(9.1%)
MOF[k]	10	(1.9%)

Demographics are reported as a number and percentage except age and obesity which are reported as median and range.

[a]Body mass index, data available on 390 patients.

[b]Alcoholism was diagnosed or patient had previously been treated for alcohol-induced disease.

[c]Plasma creatinine concentration continually more than 170 μmol L^{-1} (five patients underwent chronic dialysis treatment).

[d]Continuous medication for cardiovascular disease (including hypertension and arteriosclerosis).

[e]Continuous systemic cortisone treatment (daily dose > 10 mg oral prednisolone).

[f]Intensive care unit.

[g]Systolic blood pressure < 90 mmHg or a reduction of 40 mmHg from baseline. No response to 500 mL intravenous fluid replacement.

[h]Disseminated intravascular coagulation.

[i]Glasgow coma scale < 15.

[j]Continuous positive airway pressure/ bilevel positive airway pressure.

[k]Multi-organ failure.

Table 2 *The distribution of emergency room patients stratified by diagnosis group into low or high FURIN levels.*

Diagnosis group	Criteria	FURIN < 370 pg/ml (N = 429) n	percentage	FURIN ≥ 370 pg/ml (N = 108) n	percentage
1. No SIRS, no bacterial infection (N = 59)	Patients with no SIRS[a] (less than two SIRS criteria +/- 24 hours) or documented[b] or probable[c] bacterial infection	46	(78.0%)	13	(22.0%)
2. Bacterial infection, no SIRS (N = 67)	Patients with documental or probable bacterial infection, but no SIRS (less than two SIRS criteria +/- 24 hours)	57	(85.1%)	10	(14.9%)
3. SIRS, no bacterial infection (N = 54)	Patients with SIRS (at least two SIRS criteria +/- 24 hours), but no documental or probable bacterial infection	45	(83.3%)	9	(16.7%)
4. Sepsis (N = 308)	Patients with sepsis (SIRS and documented or probable bacterial infection but no dysfunction due to sepsis)	242	(78.6%)	66	(21.4%)
5. Severe sepsis (N = 49)	Patients with severe sepsis (sepsis with signs of organ failure, i.e. distrubted perfusion, metabolic acidosis, oliguria or neurological disorders)	39	(79.6%)	10	(20.4%)

Differences between five groups were studied using the Chi-Square test ($P = 0.737$).

[a]Systemic inflammatory response syndrome (SIRS): at least two of the following conditions. 1. Temperature > 38°C or < 36°C; 2. Heart rate >90 beats per min; 3. Respiratory rate > 20 breaths per min or partial pressure of carbon dioxide in arterial blood [PaCO2] < 32 mmHg (4.3 kPA). 4. White blood cell count > 12 x 109 L-1 or > 10% immature (band) forms).

[b]Documented bacterial infection: microbiologically confirmed bacterial infection (either pathogenic bacterial growth in blood culture or in normally sterile tissue or the same usually less pathogenic bacterium, for example, *Staphylococcus epidermidis*, in two different samples).

[c]Probable bacterial infection: a clinician suspected bacterial infection and either infection focus was confirmed or antimicrobial treatment was started and the response to treatment supported bacterial infection.

We next assessed if high FURIN level in plasma associated with patients' demographic characteristics or infection-associated clinical parameters (Table 3). Although elevated FURIN levels have been found in malignancies and atherosclerosis [11, 13, 25], there were no statistically significant associations with high FURIN plasma levels and the prevalence of solid cancer (P = 0.606) or cardiovascular diseases (P = 0.204). However, in keeping with the elevated FURIN expression in inflamed tissues in RA [19], FURIN plasma concentrations over 370 pg/ml associated highly significantly with a diagnosed rheumatic disease. Specifically, only 7.0% of the patients in the group with lower levels of FURIN were suffering from rheumatic diseases in contrast to 18.5% in the high FURIN level group (P < 0.001). The plasma level of FURIN was over 370 pg/ml in 40% of arthritis patients compared to only 18 % in the cohort without rheumatic disease (data not shown). Interestingly, albeit tobacco smoking places a burden on the immune system, and could thus be envisioned to increase FURIN expression through the immune cell activation, we also found a significant association between current smokers and low FURIN plasma levels (P = 0.034).

In contrast, during the first 28 days FURIN plasma levels did not significantly associate with any of the investigated clinical parameters including multi-organ failure (P = 0.421) or the occurrence of sepsis (P = 0.957); also there was no statistically significant connection to a 28-day case fatality when examining patients with sepsis as a direct cause of dead or a contributing factor on patients decease (P = 0.463). In conclusion, the current data imply that assessing FURIN plasma levels does not have value as a diagnostic marker for patients with a suspected infection.

Table 3. *The distribution of patients' demographic characteristics and clinical parameters into the two FURIN levels (N = 537).*

	FURIN < 370 pg/ml (N = 429)		FURIN ≥ 370 pg/ml (N = 108)		
	N	Percentage	N	Percentage	*P*-value
Characteristic					
Age, median (range)	63.82 (18 - 100)		66.38 (20 - 94)		0.618
Gender (male)	246	(57.35%)	64	(59.3%)	0.719
Obesity (BMI[a] ≥ 30 kg/m^2), median (range)	27.20 (14.7 - 62.5)		26.67 (15.1 - 67.6)		0.320
Alcohol abuse[b]	22	(5.1%)	3	(2.8%)	0.300
Smoking (current smoker)	109	(25.4%)	17	(15.7%)	**0.034**
Diabetes (types 1 and 2)	68	(15.9%)	13	(12.0%)	0.322
Solid cancer	64	(14.9%)	14	(13.0%)	0.606
Malignancy (solid or haematological)	79	(18.4%)	16	(14.8%)	0.381
Rheumatic disease	30	(7.0%)	20	(18.5%)	**<0.001**
Chronic renal insufficiency[c]	16	(3.7%)	2	(1.9%)	0.333
Cardiovascular disease[d]	225	(52.4%)	64	(59.3%)	0.204
Continuous cortisone treatment[e]	42	(9.8%)	17	(15.7%)	0.077
Clinical parameters (d 0-28)					
Case fatality (d 28)	28	(6.5%)	5	(4.6%)	0.463
Case fatality (d 90)	49	(11.4%)	9	(8.3%)	0.355
Case fatality (1 year)	90	(21.0%)	22	(20.4%)	0.889
ICU[f] stay needed	30	(7.0%)	12	(11.1%)	0.154
Hypotension[g]	23	(5.4%)	5	(4.6%)	0.760
DIC[h]	7	(1.6%)	1	(0.9%)	0.588
Decreased GCS[i]	50	(12.2%)	10	(9.6%)	0.460
Mechanical ventilation	11	(2.6%)	3	(2.8%)	0.901
C-PAP/bi-PAP[j]	16	(3.7%)	6	(5.6%)	0.392
Sepsis + organ dysfunction	39	(9.1%)	10	(9.3%)	0.957
MOF[k]	9	(2.1%)	1	(0.9%)	0.421

Demographics are reported as a number and percentage in the particular concentration of FURIN
Except age and obesity which are reported as median and range.

[a]Body mass index, data available on 390 patients.

[b]Alcoholism was diagnosed or patient had previously been treated for alcohol-induced disease.

[c]Plasma creatinine concentration continually more than 170 μmol L^{-1} (five patients underwent chronic dialysis treatment).

[d]Continuous medication for cardiovascular disease (including hypertension and arteriosclerosis).

[e]Continuous systemic cortisone treatment (daily dose > 10 mg oral prednisolone).

[f]Instesive care unit.

[g]Systolic blood pressure < 90 mmHg or a reduction of 40 mmHg from baseline.
No response to 500 mL intravenous fluid replacement.

[h]Disseminated intravascular coagulation.

[i]Glasgow coma scale < 15.

[j]Continuous positive airway pressure/ bi-level positive airway pressure.

[k]Multi-organ failure.

To investigate whether FURIN plasma levels could associate with the gram staining results of the blood culture samples, we divided the study population into four groups with the alternatives of no growth, gram-positive, gram-negative and mixed infection (Table 4); no significant differences in FURIN levels were observed (P = 0.351). To explore the more accurate distribution of various bacterial species into the two FURIN plasma levels the blood culture results were further divided into the gram-positive and gram-negative bacterial species (Table 5). Gram-negative *Escherichia coli* (n = 11) and gram-positive *Streptococcus pneumoniae* (n = 10) were noticeably the most common causes of a systemic bacterial infection among our study cohort. However, the findings again indicated that FURIN plasma levels measured simultaneously with the blood culture sampling is not informative considering the bacterial strain causing the infection.

Table 4 *The distribution of the Gram-staining of bacteria in blood culture findings into the FURIN levels.*

	FURIN < 370 pg/ml (N = 429)		FURIN ≥ 370 pg/ml (N = 108)	
Blood culture finding	N	Percentage	N	Percentage
No growth (N = 485)	389	(80.2%)	96	(19.8%)
Gram-positive (N = 28)	20	(71.4%)	8	(28.6%)
Gram-negative (N = 16)	12	(75.0%)	4	(25.0%)
Mixed (N = 7)	7	(100.0%)	0	(0.0%)

Table 5 *The distribution of different bacterial species measured from blood cultures into two FURIN levels*

	FURIN < 370 pg/ml N	FURIN ≥ 370 pg/ml N	Total
Gram-positive bacteria			
Stafylococcus aureus	3	1	4
Other Stafylococcus	2	2	4
Streptococcus pneumoniae	8	2	10
Other Streptococcus	4	1	5
Listeria monocytogenes	0	1	1
Anaerobic gram-positive bacteria/bacills	2	1	3
Gram-negative bacteria			
Escherichia coli	9	2	11
Pseudomonas	1	0	1
Other Enterobacterium *	3	0	3
Neisseria meningitidis	0	1	1
Klebsiella	4	1	5
Anaerobic gram-negative bacteria/bacills	1	0	1

Differences between values in the table were studied using the Chi-Square test ($P = 0.499$).
* *Salmonella, Morganella, Proteus, Pantotea*

To estimate the potential use of FURIN as a marker for a patient's prognosis in predicting the severity and the case fatality, we constructed two survival curves. First, we examined the overall case fatality during the one-year study period (Figure 1). The plasma level of FURIN failed to prognosticate the case fatality of an infection in a one-year lasting follow-up period ($P = 0.742$). Finally, we wanted to clarify the possible prognostic value of FURIN concentrations especially among the patients who had been diagnosed with sepsis at the emergency room. According to the fact that basically all of the sepsis-related deaths occur in the first four weeks since the symptoms had started, we limited the follow-up time into 28 days. The cases where sepsis was not a direct cause of dead or did not contribute to patient's decease were censored (n = 13) from the analysis (Figure 2). The statistical significance was not found between the survival probability of septic patients and FURIN concentration level ($P = 0.898$).

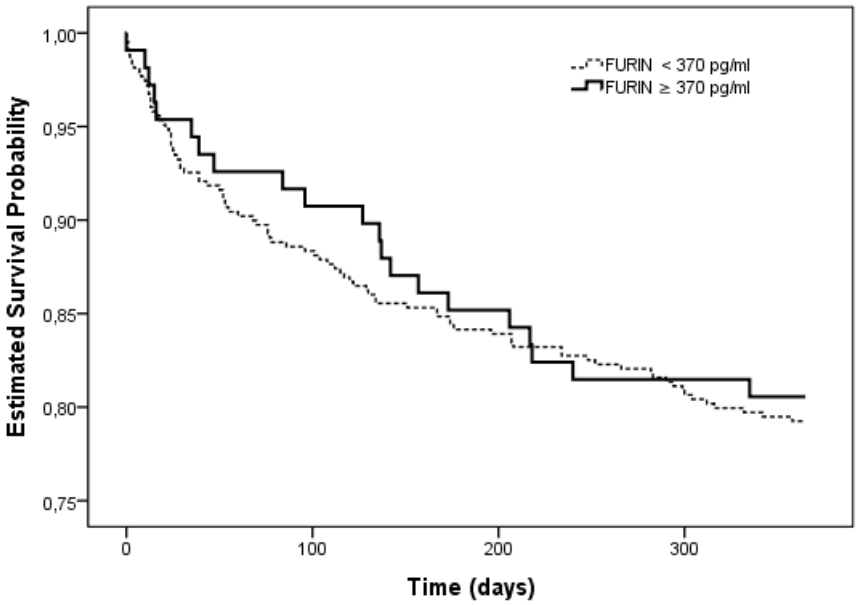

Figure 1. The survival curve measuring the correlation between low (N = 429) and high (N = 108) FURIN levels and the case fatality among the entire study population explored during the one-year follow-up (P = 0.742).

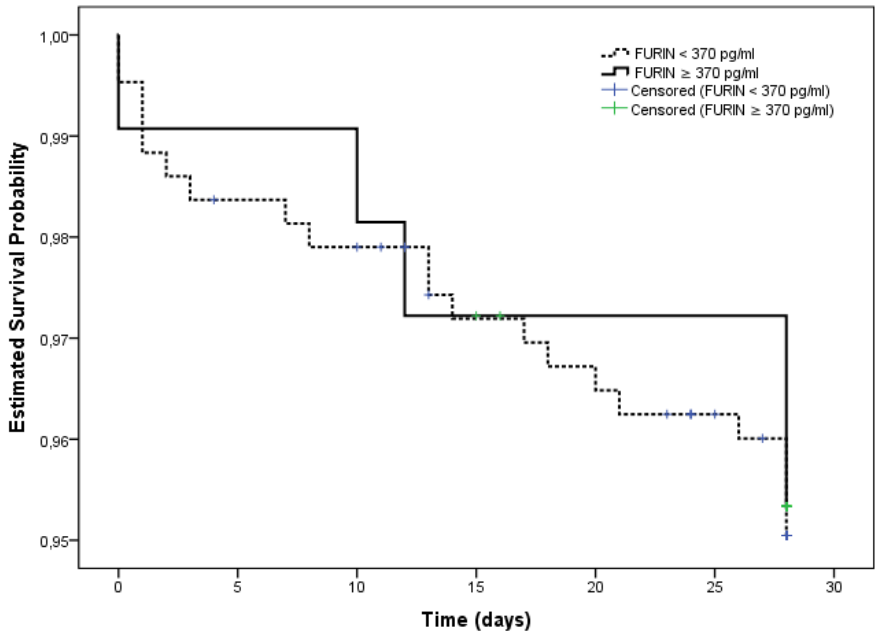

Figure 2. The survival curve measuring correlation between low (N = 429) and high (N = 108) FURIN levels and the case fatality among the patients with the septic infection explored during the 28-day follow-up (P = 0.898). Deaths for which the sepsis found in the emergency room did not influence or was not the direct the expiration, are censored from the curve (N = 13).

4. DISCUSSION

We examined the usability of the ubiquitously expressed proprotein convertase FURIN as an infection biomarker by determining its plasma concentrations in the study cohort of 537 patients admitted to the emergency room with a suspected infection. The present results revealed that FURIN cannot be used as a diagnostic biomarker at the emergency room in the early phase of an infection, even though it has earlier been associated with various inflammatory conditions [12, 13, 14, 16, 17, 18, 20]. There were also no statistically significant associations between the FURIN plasma levels and the gram staining or the bacterial species of the blood cultures. The concentration of FURIN neither associated with the case fatality or the severity of the infection in our study cohort. In contrast, we found that patients with a rheumatic disease and patients that were not currently smokers had significantly more frequently high plasma concentrations of FURIN.

The strong association of the elevated plasma FURIN in patients with a rheumatic disease is consistent with the earlier findings showing upregulated FURIN in the inflamed joints of RA patients [19]. The role of FURIN in autoimmune diseases is not fully understood, but its expression in T cells is critical for the functional maturation of pro-TGF-β1 and adequate function of CD4+Foxp3+ T regulatory cell dependent immune tolerance [16]. Moreover, administration of the recombinant FURIN alleviates the inflammation in an experimental collagen-induced arthritis (CIA) mouse model [19]. Theoretically elevated FURIN levels in plasma could thus reflect an attempt to harness overly active host defense by the activation of anti-inflammatory responses. However, as T-cell-expressed FURIN also inhibits Th2 responses and promotes Th1-type inflammation further studies are clearly required to fully understand the immunoregulatory role of FURIN in plasma [15]. In any case our current finding calls for a further assessment of the value of plasma FURIN levels as a biomarker in autoimmune diseases.

A statistically significant association was also found between the FURIN concentrations below 370 pg/ml and the habit of smoking. This is somewhat counterintuitive as smoking is generally regarded to evoke a chronic inflammatory state in the body involving the expansion in the quantity of proinflammatory cytokines [26]. One possible explanation could be that tobacco-related substances in plasma interfere with the FURIN measurement, and thus falsify the data. Therefore, analyzing FURIN mRNA or protein expression directly in the lungs could give more reliable data on the effect of smoking on FURIN levels.

To our knowledge, this was the first study assessing the usefulness of proprotein convertase FURIN as a diagnostic biomarker for the infections. The strengths of our study were a randomly selected and comprehensive study cohort, a high quality of information collection and the follow-up together with the broad data on patients' demographic and clinical characteristics. The wide data including the information starting from the underlying diseases and medications as well as received treatment during the hospital stay and rehabilitation occurred within one year study time, allowed us to undergo multifarious statistical analysis. The information bias and random error were minimized by taking the plasma samples for the study simultaneously with the samples taken for the diagnostic tests and defining the exact timing for the interviews. This reduced the potential occurrence of both the selection and the information bias making the presented results largely generalizable to the infectious patients in the Finnish population.

The sensitivity of the FURIN ELISA kit did not allow measurement of low FURIN concentrations, but forced us to stratify the samples into low and high FURIN groups. The transformation of the variable from scale to ordinal caused a loss of information and moreover led to the reduction of a statistical power of our data. In addition to us, also other research teams have reported the sensitivity of FURIN antibody assays as problematic [27]. A more sensitive method for the determination of proprotein convertases in plasma could be a direct measurement of the FURIN´s activity using a fluorogenic substrate [28]. The downside is that the concerned technique does not distinguish PCSK enzymes from each other.

To determine the FURIN plasma levels in a healthy population we performed a small-scale pilot study with six healthy volunteers (data not shown). All these samples showed FURIN levels below the cut-off used in this study, but a larger cohort and a more sensitive measurement technique is clearly needed to obtain reliable information on the range of normal plasma FURIN. This may also result in reinterpretation of the current data; if the starting levels of FURIN in healthy individuals are highly variable the potential rise during the infection would have remained unnoticed even if it occurred consistently with every patient. Also, due to lack of sensitivity of the used ELISA kit it cannot be excluded that infection-associated changes below 370 pg/ml may have a diagnostic value.

In conclusion, we have shown that plasma FURIN levels assessed with the current method can not be used as a diagnostic biomarker in patients with suspected infection and fail to predict case fatality or severity. However, even a low sensitivity ELISA measurement implies that plasma FURIN levels may associate with autoimmunity.

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